Heterogeneity of the Sensitivity of Vesicular Stomatitis Virus to Interferons

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Twelve cloned viruses were randomly isolated from original (uncloned) vesicular stomatitis virus (VSV), and their sensitivities to mouse and human interferons were examined. When the interferon sensitivities of these various VSVs were compared by the plaque reduction method in L cells, virus 3 was found to be sevenfold more sensitive than virus 11, and the interferon sensitivity of the original (uncloned) virus was intermediate. The present study shows that uncloned VSV Indiana strain is a mixture of viruses that have different sensitivities to interferon. The slope of the dose-response curve of original (uncloned) virus to mouse interferon was less steep than those of cloned viruses. Virus 11, which was the least sensitive to mouse interferon, was relatively sensitive to human interferon. There was no correlation between the sensitivities of virus clones to mouse interferon and their sensitivities to human interferon. When the interferon sensitivities were tested by various assay methods (plaque reduction, yield reduction, and cytopathic effect inhibition), the ranking of the interferon sensitivities of different viruses was not changed. These results indicate that the relative sensitivity of a virus to interferon is determined by the host cells in which the tests are performed, but not by assay method used.

Interferons, which are proteins synthesized by a variety of animal cells in response to viral infection as well as to many nonviral stimuli, inhibit the multiplication of a wide range of unrelated deoxyribonucleic acid and ribonucleic acid viruses. Wide variations in the responses of different viruses to inhibition by interferon have suggested a ranking of viruses based on their sensitivity to this inhibitor (1). The arbovirus group is usually considered most sensitive; vesicular stomatitis virus (VSV), vaccinia virus, and other poxviruses are of intermediate sensitivity; and Newcastle disease virus (NDV), herpes simplex virus, and cytomegalovirus are the least sensitive (3, 8, 11). However, when a virus is referred to as interferon sensitive or interferon insensitive, the species of interferon involved must be stated. Stewart et al. (8) reported that certain viruses were relatively sensitive to some interferons but relatively insensitive to interferons from other species.

In this study, 12 cloned viruses were randomly isolated from original (uncloned) VSV (Indiana strain), and their sensitivities to mouse and human interferons were examined.

MATERIALS AND METHODS

VSV. The Indiana strain of VSV was kindly supplied by E. De Maeyer, Institut du Radium, Univer-

sité de Paris-Sud, Orsay, France. The virus was propagated in L cells by inoculation at a multiplicity of 0.01 to 0.001 plaque-forming units (PFU)/cell.

Cell cultures and media. Cells used in this study were mouse L cells and human MRC-5 cells. L cells were grown in minimum essential medium (MEM) supplemented with 10% calf serum, and MRC-5 cells were grown in basal medium fortified with 10% fetal calf serum.

Preparation of interferons. Mouse interferon was produced in confluent cultures of C_{243} cells stimulated with polyinosinic · polycytidylic acid (30 µg/ml), and diethylaminoethyl-dextran (100 µg/ml) was prepared as described previously (9). Human interferon was prepared from MRC-5 cells infected with NDV inactivated by ultraviolet irradiation. To eliminate infectious virus, interferon preparations induced by ultraviolet-irradiated NDV were dialyzed overnight at pH 3 and redialyzed to neutrality against MEM.

Interferon titration. Interferon was assayed by the plaque reduction, cytopathic effect (CPE) inhibition, and yield reduction methods.

(i) Plaque reduction method (5). Monolayer cultures of L and MRC-5 cells in 35-mm plates were incubated with 1.5 ml of a threefold dilution. After 20 h of incubation, the cells were drained and challenged with about 50 PFU of VSV. After 1 h of adsorption at 37°C, the culture was overlayed with 0.6% agarose in MEM. On day 2 of virus challenge, the cells were stained with neutral red and plaques were counted. Interferon titers were expressed as the reciprocals of dilutions causing a 50% plaque count reduction.

(ii) CPE inhibition method. A modification of the semi-micro method described by Havell and Vilcek (4) was used. Individual wells of microtest tissue cultures plates (Nunclon) were first filled with 100 μl of MEM without serum. To prepare a series of twofold dilutions, 100 μl of the titrated sample was added to the first well containing the same volume of medium; serial dilutions were prepared by transferring 100 μl of the mixtures with a micropipette. Each well was then seeded with 30,000 L cells in 50 μl of MEM supplemented with 5% calf serum. After 20 h of incubation at 37°C in a CO_2 incubator, each well received 100 50% tissue culture-infectious doses of VSV in 50 μl of MEM. Several wells on each plate served as virus controls and cell controls. The titrations were scored microscopically 24 to 48 h after virus inoculation, when the virus controls showed complete destruction by the virus. The highest dilution of the titrated sample causing at least 50% protection was considered the end point. Each sample was titrated in duplicate.

(iii) Yield reduction method. Monolayer cultures of L cells were incubated in the presence of serial dilutions of interferon in MEM without serum for 20 h at 37°C. After the interferon dilutions were removed, the cells were infected with VSV at an input multiplicity of infection of 5, and adsorption at 37°C was carried out for 60 min. The cell cultures were washed three times to remove unadsorbed virus, refed with medium, and reincubated at 37°C. Fluids from cultures infected with VSV were harvested 20 h after infection and assayed for PFU on L-cell monolayers. One unit of interferon was defined as the dilution of the interferon sample that reduced the VSV yield by 50% compared with controls.

RESULTS

Relative sensitivities of viruses to interferons: plaque reduction method. Twelve cloned viruses of VSV were randomly isolated by plaque purification in L cells and were then propagated in L cells by inoculation at a multiplicity of 0.01 to 0.001 PFU/cell. After incubation at 37°C for 20 h, the medium was collected, centrifuged for 10 min at 2,000 rpm, and stored at −80°C.

To determine the sensitivities of the 12 cloned viruses of VSV to the mouse interferon, plaque reduction assays were performed simultaneously in L cells. The average percentage of reduction of plaques in three separate determinations was plotted for each dilution of interferon, and the 50% end point for each virus was determined graphically. Virus 3 was the most sensitive of the 12 cloned viruses to mouse interferon (Table 1). Virus 11 was seven times less sensitive than virus 3 (Table 1).

Human interferon was assayed against the same viruses. Virus 12 was the most sensitive, and virus 2 was 10 times less sensitive than virus 12. The relative sensitivities to human interferon showed an interesting contrast to those found with mouse interferon. Virus 11, which

TABLE 1. *Heterogeneity of the sensitivity of VSV to interferons*

Virus[a] (clone no.)	Interferon titer[b] (mean) in:	
	L cells	MRC-5 cells
1	1,600	1,900
2	4,000	1,500
3	6,600	3,200
4	1,700	ND[c]
5	3,200	ND
6	1,900; 2,663	ND; 4,278
7	2,700	1,700
8	3,900	ND
9	1,700	ND
10	1,900	2,900
11	960	3,250
12	1,800	15,500
Original VSV (uncloned)	2,500	7,600

[a] Twelve cloned viruses of VSV were randomly isolated from one strain of VSV (Indiana strain) by plaque purification in L cells.

[b] Assayed by the plaque reduction method, using L and MRC-5 cells and VSV. The same interferon preparation was assayed by using the 12 cloned VSVs and original (uncloned) VSV. Mouse interferon assays were performed in L cells, and human interferon assays were performed in MRC-5 cells.

[c] ND, Not done.

was the least sensitive to mouse interferon, was relatively sensitive to human interferon. There was no correlation of the sensitivities of viruses to mouse interferon and to human interferon (Fig. 1). The original (uncloned) virus showed intermediate sensitivity to both interferons.

Figure 2 shows dose-response curves comparing the effects at each dilution of mouse interferon on plaque formation by viruses 3, 7, and 11 and the original (uncloned) virus. The slope of the dose-response curve of the original (uncloned) virus was very shallow compared with those of cloned viruses.

Relative sensitivities of viruses to mouse interferon: various interferon assay methods. Experiments were designed to investigate whether the ranking of interferon sensitivity of virus in the same cell system depended on the assay method used. To determine the sensitivities of viruses to mouse interferon, the plaque reduction, CPE inhibition, and yield reduction assays were performed simultaneously against the original (uncloned) virus, virus 3, which was the most sensitive to mouse interferon (plaque reduction method), and virus 11, the least sensitive virus to mouse interferon (plaque reduction method). Virus 3 was the most sensitive and virus 11 was the least sensitive to mouse interferon by all methods tested (Fig. 3 and 4).

Table 2 shows the same characteristics of

these viruses. There was little difference among these viruses in the mean diameter of plaques. However, the plaque sizes of original (uncloned) VSV varied. Interferon induction by these viruses in L cells was not detected. The single-cycle yields of these viruses were not much different in L cells.

DISCUSSION

The present study shows that 12 cloned viruses, randomly isolated from original (uncloned) VSV, had different interferon sensitivities. When these interferon sensitivities were compared by the plaque reduction method in L cells, virus 3 was sevenfold more sensitive than virus 11, and the interferon sensitivity of the original (uncloned) virus was intermediate.

Wide variations in the responses of different viruses to inhibition by interferon have been suggested by many investigators (see reference 1). When the sensitivities of NDV and VSV to interferon were compared by the plaque reduction method in primary chicken embryo cell cultures, NDV was found to be 45-fold more resistant than VSV (3). Vaccinia virus was the most sensitive of the five viruses (vaccinia virus, VSV, Semliki Forest virus, Sindbis small-plaque strain, and Sindbis large-plaque strain) to mouse and hamster interferons (8). The present study shows that uncloned VSV Indiana strain is a mixture of viruses that have different sensitivities to interferon. Wagner et al. (10) found that two variants of VSV, small-plaque and large-plaque variants, had different sensitivities to mouse interferon. The cloned lines of VSV used in this study were randomly isolated from original (uncloned) VSV and produced plaques
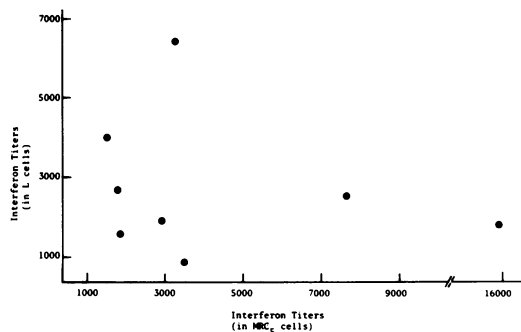

FIG. 1. Correlation of the sensitivities of various VSVs to mouse interferon and those to human interferon. Interferon titers were assayed by the plaque reduction method, using L and MRC-5 cells and VSV. The same interferon preparations were assayed by using various viruses. Mouse interferon assays were performed in L cells, and human interferon assays were performed in MRC-5 cells.
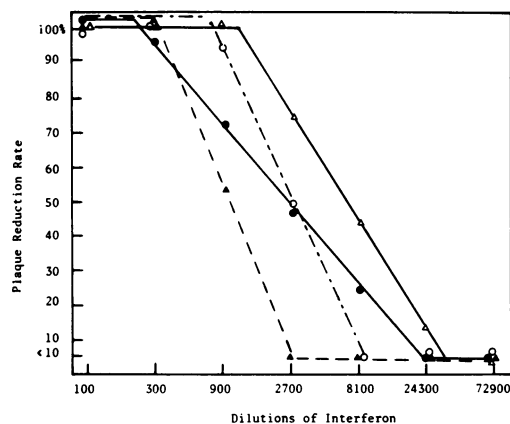

FIG. 2. Dose-response curves comparing the effects at each dilution of mouse interferon on plaque formation by virus 3 (△), virus 7 (○), virus 11 (▲), and the original virus (●).

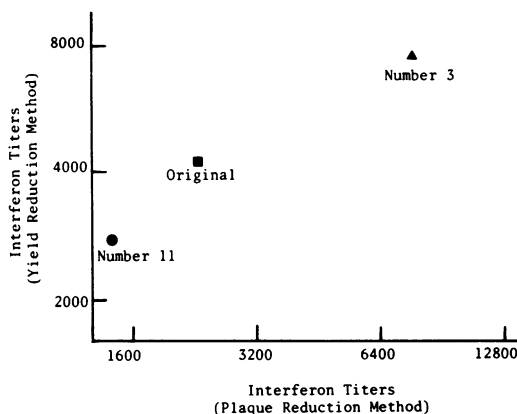FIG. 3. Plaque reduction and the yield reduction assays were performed simultaneously against the original virus (■), virus 3 (▲), and virus 11 (●).

of about the same diameter, although the plaque sizes of original (uncloned) VSV varied. Simon et al. (7) have recently reported that interferon-sensitive (is) mutants of mengovirus can be isolated by using a mutagen (HNO_2). One of these mutants, is-1, grows normally in control L cells, but in cells pretreated with interferon, its yield is at least 10-fold lower than that of the wild type (is$^+$).

The slope of the dose-response curve of original (uncloned) virus to mouse interferon was very shallow when compared with those of cloned viruses. Y. Nishiyama (Nagoya University) has observed the same phenomenon (personal communication). This phenomenon can be explained by the experimental finding that original (uncloned) VSV is a mixture of viruses that have different sensitivities to interferon.
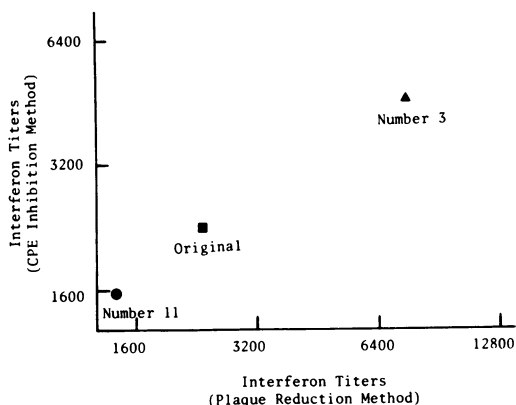
FIG. 4. *Plaque reduction and the CPE inhibition assays were performed simultaneously against the original virus (■), virus 3 (▲), and virus 11 (●).*

TABLE 2. *Some characteristics of the original and cloned VSV*

Virus	Yield of virus[a] (PFU/ml)	Plaque size[b] (mm)	Interferon sensitivity[c]	Interferon inducibility[d]
Original	1.1×10^8	1.9 ± 1.5	1	<3
No. 3	0.92×10^8	2.0 ± 0.2	2.6	<3
No. 11	1.0×10^8	2.0 ± 0.2	0.38	<3

[a] Monolayer cultures of L cells were infected with various viruses at an input multiplicity of infection of 10 PFU/cell. After adsorption for 1 h, the cultures were washed three times with MEM and incubated with MEM without serum at 37°C. Virus yields at 20 h after infection were determined. Infectivity titers of VSV in the culture fluids were determined by the plaque method, using monolayers of L cells.

[b] Plaque size at 2 days after infection was measured.

[c] Interferon sensitivity of original VSV to mouse interferon was taken as 1.

[d] Interferon activities of the culture fluids of L cells infected with various VSVs were measured.

The theoretical percentage of plaque reduction can be expressed as follows (2): percent reduction = $100(1 - ae^{-bI})$, where I is the interferon dose, a is the parameter determining the slope of the dose-response curve, and b varies with the interferon sensitivity of the virus or cell. Figure 5 is a theoretical plot in which the percentage of plaque reduction is plotted against values of interferon for two cloned virus (A and B). Let us assume that an actual virus population consists of these viruses, which vary in sensitivity to interferon by a factor of nine, and that the two virus types are present in the population in equal numbers. The slopes of lines A and B are about 105.3% per decade, which is calculated from Fig. 2. The percentage of reduction resulting from such a mixture would be: $50(1 - ae^{-I}) + 50(1 - ae^{-9I})$. Curve C (Fig. 5) would result with a slope of 52.4% per decade. If interferon
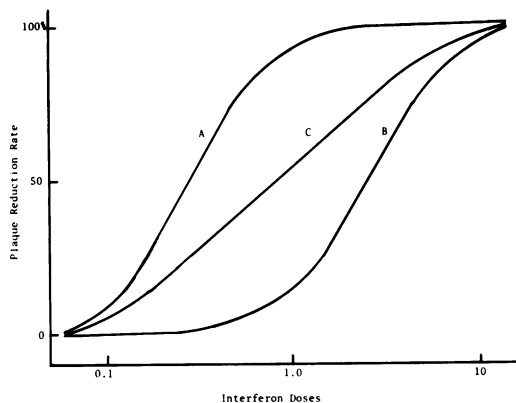

FIG. 5. *Plot of two theoretical virus types in which the percentage of plaque reduction is plotted against values of I (interferon dose). The constant b varies by a factor of nine (i.e., for line A, b = 9, and for line B, b = 1). Line C would result from an equal mixture of the two virus types.*

titration is performed with a cloned line of VSV instead of with uncloned virus, the steep slope of the dose-response curve should permit a more accurate determination of the 50% end point.

Stewart et al. (8) found that each species of interferon has a characteristic spectrum of activity against viruses. Although virus A may be much more sensitive than virus B to one species of interferon, the reverse situation may be found with another interferon species. Virus 11, which was the least sensitive to mouse interferon, was relatively sensitive to human interferon. There was no correlation between the sensitivities of viruses to mouse interferon and those to human interferon. The original (uncloned) virus showed intermediate sensitivity to both interferons. When the interferon sensitivity was tested by various interferon assay methods, the ranking of interferon sensitivity of different viruses was not changed. These results indicate that the relative sensitivity of a virus to interferon is determined by the host cell in which the tests are performed and/or the type of interferon used, but not by assay method used.

This study shows that it is difficult to define a virus as interferon sensitive or interferon resistant.

LITERATURE CITED

1. **Finter, N. B. (ed.).** 1973. Interferons and interferon inducers. *In* Frontiers of biology, vol. 2, p. 135. American Elsevier, New York.
2. **Gifford, G. E., and A. L. Koch.** 1969. The interferon dose response curve and its possible significance. J. Theor. Biol. **22**:271–283.
3. **Hallum, J. V., R. H. Thacore, and J. S. Youngner.** 1970. Factors affecting the sensitivity of different viruses to interferon. J. Virol. **6**:156–162.

4. Havell, E. A., and J. Vilcek. 1972. Production of high-titered interferon in cultures of human diploid cells. Antimicrob. Agents Chemother. 2:476–484.

5. Ito, Y., Y. Nisiyama, K. Shimokata, Y. Kimura, I. Nagata, and A. Kunii. Interferon-producing capacity of germfree mice. Infect. Immun. 13:332–336.

6. Lomniczi, B. 1973. Studies of interferon production and interferon sensitivity of different strains of Newcastle Disease Virus. J. Gen. Virol. 21:305–313.

7. Simon, E. H., T. Kung, T. Koh, and P. Brandman. 1976. Interferon-sensitive mutants of mengovirus. 1. Isolation and biological characterization. Virology 69:727–736.

8. Stewart, W. E., II, W. D. Scott, and S. E. Sulkin. 1969.

9. Thang, M. N., C. Thang, E. De Maeyer, and L. Montagnier. 1975. Biosynthesis of mouse interferon by translation of its messenger RNA in a cell-free system. Proc. Natl. Acad. Sci. U.S.A. 72:3975–3977.

10. Wagner, R. R., A. H. Levy, R. M. Snyder, G. A. Ratchliff, and D. F. Hyatt. 1963. Biologic properties of two plaque variants of vesicular stomatitis virus (Indiana serotype). J. Immunol. 91:112–121.

11. Youngner, J. S., H. R. Thacore, and M. E. Kelly. 1972. Sensitivity of ribonucleic acid and deoxyribonucleic acid viruses to different species of interferon in cell cultures. J. Virol. 10:171–178.

Relative sensitivities of viruses to different species of interferon. J. Virol. 4:147–153.